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Kcnq1 Contributes to an Adrenergic-Sensitive Steady-State K⁺ Current in Mouse Heart

Bjorn C. Knollmann^{1,2,§,†}, Syevda Sirenko^{1,†}, Qi Rong^{1,†}, Alexander N. Katchman¹, Mathew Casimiro³, Karl Pfeifer³, and Steven N. Ebert^{1,*}

¹Department of Pharmacology, Georgetown University Medical Center, Washington, DC

²Department of Medicine, Georgetown University Medical Center, Washington, DC

³Laboratory of Mammalian Genes & Development, National Institute of Child Health and Development, The National Institutes of Health, Bethesda, MD

Abstract

It has been suggested that Kcne1 subunits are required for adrenergic regulation of Kcnq1 potassium channels. However, in adult mouse hearts, which do not express Kcne1, loss of Kcnq1 causes a Long QT phenotype during adrenergic challenge, raising the possibility that native Kcnq1 currents exist and are adrenergically regulated even in absence of Kcne1. Here, we used immunoblotting and immunohistochemical staining to show that Kcnq1 protein is present in adult mouse hearts. Voltage-clamp experiments demonstrated that Kcnq1 contributes to a steady-state outward current (I_{SS}) in wild-type (*Kcnq1*^{+/+}) ventricular myocytes during isoproterenol stimulation, resulting in a significant 7.1% increase in I_{SS} density (0.43 ± 0.16 pA/pF, $p < 0.05$, $n = 15$), an effect that was absent in Kcnq1-deficient (*Kcnq1*^{-/-}) myocytes (-0.14 ± 0.13 pA/pF, $n = 17$). These results demonstrate for the first time that Kcnq1 protein is expressed in adult mouse hearts where it contributes to a β -adrenergic-induced component of I_{SS} that does not require co-assembly with Kcne1.

Keywords

Potassium channel; transgenic mouse; electrophysiology; adrenergic; heart

INTRODUCTION

The *KCNQ1* gene encodes a 6-transmembrane domain α -subunit of a K⁺ channel protein that can either form a homomeric channel or partner with single transmembrane domain β subunits encoded by the KCNE family of genes to form heteromeric channels (1-8). The biophysical properties of the KCNQ1 channel differ greatly depending upon the β -subunit with which it is co-expressed.

In the human heart, KCNQ1 is thought to primarily partner with KCNE1 to form a heteromeric channel protein that produces the slow component of the delayed rectifier current, I_{KS} (2),

[§]Present affiliation: Departments of Medicine and Pharmacology, Vanderbilt University School of Medicine, Nashville, TN

[†]These three authors contributed equally to this work.

***Corresponding Author:** Present address: Steven N. Ebert, PhD Associate Professor Burnett College of Biomedical Sciences Biomolecular Science Center University of Central Florida BMS building, Room 321/321A Orlando, FL 32816-2364 ebert@mail.ucf.edu Tel: (407) 823-4609 Fax: (407) 823-0956

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whose amplitude is markedly increased by adrenergic stimulation (1). Mutations in both the *KCNQ1* and *KCNE1* genes have been linked to Long QT Syndrome (LQTS), a disorder that predisposes individuals to increased risk of torsade de pointes ventricular arrhythmias and sudden cardiac death (3;9-11).

The role of *KCNE1* in mediating β -adrenergic regulation of *KCNQ1* is controversial: In one study (12), co-assembly of *KCNE* and *KCNQ1* was required, while other reports showed that heterologously expressed *KCNQ1* channels were responsive to adrenergic stimulation in the absence of *KCNE1* (3;13). In mice, *Kcne1* expression is strongly down-regulated during postnatal development such that little or no *Kcne1* remains in the adult mouse heart (14;15). Correspondingly, cellular electrophysiological studies did not find I_{Ks} in adult mouse cardiomyocytes (16;17).

Despite the near-absence of *Kcne1* expression and I_{Ks} in adult mouse hearts, *Kcnq1* mRNA expression remains relatively robust in the heart throughout development and into adulthood (15;18), suggesting that *Kcnq1* might play a *Kcne1*-independent role in cardiac function.

Here we compare *Kcnq1*-null mice with wild-type littermates to test the hypothesis that *Kcnq1* channels are responsive to adrenergic stimulation in native ventricular myocytes even in the absence of *Kcne1* and to resolve the issue of *Kcnq1* function in the adult mouse heart. Specifically, *Kcnq1* protein expression was examined in adult mouse hearts using immunoblotting and immunofluorescent histochemical staining techniques, where we show that *Kcnq1* protein is present in both atria and ventricles. To determine which currents were influenced by *Kcnq1*, we examined outward K^+ currents in isolated wild-type and *Kcnq1*-deficient adult cardiomyocytes. We hypothesized that since *Kcne1* is nearly absent in adult murine myocardium, any *Kcnq1*-mediated current would contribute to the steady-state outward current (I_{ss}) because the biophysical properties of I_{ss} resemble those described for *Kcne1*-independent *Kcnq1* currents described in heterologous expression systems (1;2;4;6;19).

Our results show that the β -adrenergic agonist, isoproterenol, significantly enhances I_{ss} in wild-type ventricular myocytes but has no significant effect on this current in *Kcnq1*-deficient myocytes. Thus, our data suggest that *Kcnq1* is expressed in the adult murine heart where it contributes to a β -adrenergic sensitive component of the outward steady-state K^+ current, I_{ss} .

MATERIALS AND METHODS

Materials

The anti-*Kcnq1* antibody AB5932 was obtained from Chemicon International (Temecula, CA). The anti-Dihydropyridine Receptor $\alpha 2$ (DHP $\alpha 2$) subunit antibody was obtained from Sigma Chemical Co. (St. Louis, MO). Fluorescent secondary antibodies were obtained from Jackson Immunoresearch Laboratories, Inc. (West Grove, PA). All other drugs and chemicals were purchased from Sigma Chemical Co.

Animals

Kcnq1-deficient mice were maintained as previously described (20). *Kcnq1*^{+/+} and *Kcnq1*^{-/-} mice were produced by breeding heterozygous (*Kcnq1*^{+/-}) mating pairs. All animal procedures were performed in accordance with protocols approved by the Georgetown University or by the National Institute for Child Health and Development Intramural Research Program Animal Care and Use Committees. The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Immunoblotting

Preparation of membrane-enriched extracts and western blotting procedures was performed essentially as described by Pond et al. (21). Briefly, twenty micrograms of membrane extract were separated by SDS-polyacrylamide gel electrophoresis using pre-packaged Tris-Glycine (10%) gels from Invitrogen (Carlsbad, CA). The proteins were transferred to Invitrolon™ PVDF membrane (Invitrogen), and the blots were blocked, incubated with antibody solution, and developed as described previously (22).

Immunofluorescent histochemical staining

Single and double immunofluorescent histochemical staining was performed as described previously (23;24).

Whole-cell patch-clamp recordings

Adult mouse cardiomyocytes were isolated and whole-cell patch-clamp recordings were performed at 36°C as described previously (25). Briefly, pipettes with tip resistances of 2-3 MΩ were filled with solution containing (in mmol/L): KCl 155, EGTA 14, CaCl₂ 1, HEPES 10, MgATP 5, pH 7.2. Whole cell recordings were performed in control Tyrode's solutions containing (in mmol/L): NaCl 140; KCl, 5.4; glucose, 10; MgCl₂, 1; CaCl₂, 2.0; and HEPES, 10; pH 7.4. Only cells with resting potentials more negative than -70 mV were used. After whole-cell voltage clamp was established, the extracellular solution was quickly exchanged and the steady state outward current, I_{ss}, recorded using specific voltage protocols indicated in the text. To optimize recording of I_{ss}, the extracellular solution contained 2 mM 4-aminopyridine (to block slow and fast transient outward currents, I_{K,slow} and the ultra-rapid delayed rectifier, I_{Kur}), 1 μM E-4031 (to block the rapid component of the delayed rectifier, I_{Kr}), 5 μM nifedipine and low (0.1 mM) Ca⁺⁺ (to block the L-type calcium current, I_{Ca,L}), and 0 mM Na⁺ (to abolish I_{Na}). In some experiments, chloride-free solutions were used (gluconate salts) to minimize potential interference from Cl⁻ currents. Similar results were obtained in the absence and presence of Cl⁻ (not shown). Wherever indicated in the text, isoproterenol (1 μM) was applied to the external solutions and the recording protocol was replicated following baseline measurements.

RESULTS

Kcnq1 protein expression in adult murine myocardium

Although it is well-established that Kcnq1 mRNA concentrations remain relatively robust in the adult mouse heart (15;18), protein expression has not been studied. To address this issue, we used an anti-Kcnq1 antibody to probe protein extracts from mouse hearts. The predicted molecular weight for Kcnq1 is approximately 70 kDa and anti-Kcnq1 antibody detected a band of this size in extracts prepared from *Kcnq1*^{+/+} but not from *Kcnq1*^{-/-} hearts (Fig. 1A). As a control, the blots were stripped and re-probed with an anti-DHPα2 antibody, which detected a 143 kDa band of similar intensity in both extracts. These results demonstrate that Kcnq1 protein is expressed in adult mouse hearts.

To determine where Kcnq1 is localized within the heart, we used the anti-Kcnq1 antibody to perform immunofluorescent histochemical staining. An example of these results is shown in Fig. 1, where Kcnq1 protein was detected in both atrial and ventricular myocytes in *Kcnq1*^{+/+} (Fig. 1B) but not in *Kcnq1*^{-/-} (Fig. 1C) heart sections. The atrial staining pattern typically appears to be both more intense and more uniform than that observed in the ventricular myocardium.

To explore the ventricular Kcnq1 expression in more detail, we performed coimmunofluorescent staining for sarcomeric α-actinin. As shown in Fig. 1D, Kcnq1 staining

was apparent in a “ladder-like” pattern in ventricular cardiomyocytes. Co-staining of the same section for sarcomeric α -actinin enabled identification of the Z-bands (Fig. 1E). There was substantial co-expression of *Kcnq1* and sarcomeric α -actinin in these sections, as confirmed by the yellow staining in Fig. 1F, which represents overlap of *Kcnq1* and sarcomeric α -actinin expression. These results indicate that *Kcnq1* protein is expressed in a sarcomeric-like pattern within ventricular myocytes.

Electrophysiological evaluation of I_{SS} in isolated wild type and in *Kcnq1*-deficient ventricular myocytes

In the absence of *Kcne1*, *Kcnq1* produces a rapidly-activating time-independent current that has biophysical characteristics reminiscent of those described for the steady-state current, I_{SS} , in adult mouse ventricular cells (26). I_{SS} appears to be generated by more than one type of K^+ channel protein (27). Although *Kcnq1* has not previously been identified as one of the channel proteins that contribute to I_{SS} (28), its electrophysiological characteristics in heterologous expression studies either as a homomeric channel protein or in partnership with other (non-*Kcne1*) subunits such as *Kcne2* or *Kcne3* (4;6;19) create a plausible scenario for *Kcnq1* participation in I_{SS} .

To test the hypothesis that *Kcnq1* contributes to I_{SS} , we prepared cardiomyocytes from adult *Kcnq1*^{+/+} and *Kcnq1*^{-/-} animals and recorded I_{SS} . To block potentially interfering currents, we isolated I_{SS} using inhibitors of I_{Ca} , I_{Na} , and I_K (see Materials and Methods) in combination with the voltage-clamp protocol shown in Fig. 2A. I_{SS} was not different between *Kcnq1*^{+/+} and *Kcnq1*^{-/-} myocytes (6.2 ± 0.3 vs. 6.3 ± 0.3 pA/pF, $p = \text{n.s.}$, $n = 15$ and 17 , respectively), suggesting that *Kcnq1* contributes little or none to murine I_{SS} under basal conditions.

Since the *Kcnq1* channel is known to produce enhanced current following β -adrenergic stimulation and subsequent phosphorylation by protein kinase A (PKA) in heterologous expression systems (3;16), we hypothesized that β -adrenergic stimulation would selectively increase a *Kcnq1*-dependent component of I_{SS} . To test this hypothesis, we repeated the protocol in presence of the β -adrenergic agonist, isoproterenol ($1 \mu\text{M}$). Interestingly, I_{SS} increased in presence of isoproterenol in *Kcnq1*^{+/+} myocytes, whereas isoproterenol had no effect on I_{SS} in *Kcnq1*^{-/-} myocytes (compare panels B and C, Fig. 2). As a result, average values of I_{SS} following isoproterenol challenge were significantly higher in *Kcnq1*^{-/-} myocytes compared with *Kcnq1*^{+/+} myocytes (6.2 ± 0.3 vs. 6.7 ± 0.3 pA/pF, $p < 0.05$, $n = 15$ and 17 , Fig. 2D). These data suggest that the difference in I_{SS} (ΔI_{SS}) observed in the presence versus the absence of isoproterenol can be attributed to *Kcnq1*. Average ΔI_{SS} was 0.43 ± 0.16 pA/pF in *Kcnq1*^{+/+} myocytes but -0.14 ± 0.13 pA/pF in *Kcnq1*^{-/-} myocytes ($p < 0.05$, Fig. 2E). This translates into a net increase in I_{SS} density of about 7.1% in *Kcnq1*^{+/+} myocytes and no significant change in *Kcnq1*^{-/-} myocytes. Together, these data indicate that *Kcnq1* contributes to a portion of I_{SS} that is selectively enhanced by β -adrenergic stimulation.

DISCUSSION

The function of *Kcnq1* in the adult mouse heart is controversial (16;20;29-31). The major basis for this debate appears to be the well-established down-regulation of the *Kcne1* subunit and consequent loss of I_{Ks} in adult murine myocytes compared to prenatal and early postnatal developmental stages (14;15). Unlike mice, *KCNE1* expression appears to remain relatively high in human hearts through adulthood, and I_{Ks} has been readily recorded in isolated human cardiomyocytes (32;33).

Nevertheless, we have shown that targeted disruption of *Kcnq1* (20) and introduction of a specific knock-in *Kcnq1* point mutations (31) lead to development of a Long QT phenotype in adult mice. Similar QT abnormalities have been observed in isolated perfused *Kcnq1*-deficient

adult mouse hearts following challenge with sympathomimetic drugs such as nicotine, isoproterenol, and epinephrine (30), indicating this Long QT phenotype is intrinsic to the heart itself and does not reflect extra-cardiac factors.

Kcnq1 protein expression in adult mouse myocardium

To establish that Kcnq1 protein is actually expressed in the adult mouse heart, we first used an anti-Kcnq1 antibody to perform immunoblotting experiments. Our results show that a protein of approximately 70 kDa was specifically detected in extracts from *Kcnq1*^{+/+} hearts, but was completely absent in *Kcnq1*^{-/-} hearts. Thus, our data indicate that Kcnq1 protein is present in the adult murine heart.

Consistent with these immunoblotting results, we also detected Kcnq1 protein in the adult murine heart using immunofluorescent histochemical staining techniques. With this approach, we demonstrated that Kcnq1 protein is expressed in both atria and ventricles. The staining pattern was consistent with Kcnq1 expression in both atrial and ventricular working myocardium. Interestingly, immunostaining appeared more intense within the atria compared to the ventricles, suggesting that Kcnq1 channels may be more prevalent in atrial tissue. Indeed, Temple et al. (34) speculated that atrial fibrillation observed in the KCNE1 null mice could reflect a contribution of I_{KCNQ1} alone to atrial action potentials. Alternatively, the different subcellular patterns of Kcnq1 distribution in atrial versus ventricular myocytes may have contributed to the differential staining intensities. In ventricular myocytes, Kcnq1 staining patterns largely aligned with sarcomeric structures, similar to observations made with other cardiac ion channel distribution patterns in these cells (35-37), including Kcnq1 in rat ventricular myocytes (38). The more diffuse pattern observed in atrial myocytes may reflect the lack of well-developed sarcomeric and t-tubule structures in these cells (39).

Kcnq1 contributes to a β -adrenergic sensitive component of I_{SS}

Since there is no I_{Ks} present in adult murine ventricular myocytes, a role for Kcnq1 in these cells has not previously been identified. We hypothesized that Kcnq1 contributes to the steady-state outward K⁺ current, I_{SS}, because of the similar electrophysiological properties of I_{SS} in isolated ventricular myocytes (28) and I_{KCNQ1} in transfected cells. Both currents display rapidly activating kinetics and do not inactivate. To test this hypothesis, we evaluated I_{SS} in ventricular myocytes isolated from *Kcnq1*^{+/+} and *Kcnq1*^{-/-} hearts. Under control conditions, no significant differences in I_{SS} densities were observed between wild-type and mutant myocytes, thereby indicating that Kcnq1 may not contribute significantly to repolarization at baseline. In the presence of isoproterenol, however, a significant increase in I_{SS} density was observed exclusively *Kcnq1*^{+/+} myocytes. Since the isoproterenol-induced increase in I_{SS} was dependent on the presence of Kcnq1, the logical conclusion is that endogenous Kcnq1 channels mediate the increased I_{SS} densities observed in the presence of isoproterenol.

It is clear from previous studies (3;13;16) that Kcnq1 itself is the target of PKA-mediated phosphorylation, and that I_{KCNQ1} can be enhanced by PKA or forskolin in the absence of KCNE1. However, because these previous studies were performed in heterologous expression systems, it has not been previously determined if endogenous Kcnq1 could be regulated by adrenergic hormones in the absence of Kcne1. At present, we cannot conclude that Kcnq1 is acting alone (*i.e.*, homomeric Kcnq1 channels leading to I_{Kcnq1}) versus partnership with one or more non-Kcne1 subunits (*e.g.*, Kcne2 or Kcne3) (6). We can, however, conclude that Kcnq1 channel proteins are expressed in the adult mouse heart where they contribute to steady-state repolarizing currents during β -adrenergic receptor stimulation.

These results demonstrate for the first time that endogenously expressed Kcnq1 contributes to a β -adrenergic-responsive current other than I_{Ks} (and therefore independent of Kcne1) in ventricular cardiomyocytes.

Study limitations

One caveat of our work is that the findings may be specific to mice and other small rodents that display marked differences in cardiac electrophysiology compared to humans. The developmental down-regulation of Kcne1 in mice and rats likely contributes to the lack of I_{Ks} in the adult myocardium of these species, whereas I_{Ks} has been readily detected in larger mammalian species, including humans (32;33). It is not clear, however, that I_{Ks} is the only important current to which KCNQ1 contributes to even in hearts where I_{Ks} is known to be present. Indeed, a recent study by Lundquist et al. (40) showed that multiple KCNE subunits are expressed in different regions of human myocardium, and that co-expression of other KCNE(2-5) subunits can significantly affect KCNQ1 currents even when KCNE1 is also present. Furthermore, Dun and Boyden (41) recently showed that KCNQ1 contributed to steady state-like (non- I_{Ks}) currents in canine ventricular myocytes isolated from post-infarcted hearts. Clearly, additional work is needed to determine the molecular constituency and physiological correlates of cardiac channels containing Kcnq1 in humans and other species.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Identification of Kcnq1 protein in adult mouse heart

(A) Western blot assays were performed using proteins extracted from *Kcnq1*^{+/+} and *Kcnq1*^{-/-} adult mouse hearts. The blot was initially probed with an anti-Kcnq1 antibody, and then stripped and re-probed with an anti-DHPα2 antibody. (B&C) Localization of Kcnq1 protein in adult mouse hearts using immunofluorescent histochemical staining. Sections from *Kcnq1*^{+/+} and *Kcnq1*^{-/-} hearts are shown in panels B and C, respectively. Atrial tissue is in the northwest portion while ventricular tissue is in the southeast portion of each panel. (D-F) Co-immunofluorescent staining in an adult mouse (*Kcnq1*^{+/+}) ventricular muscle tissue section for (D) Kcnq1, (E) sarcomeric α-actinin, and (F) an overlay of these images. Co-expression is indicated by the yellow regions indicating overlapping staining for Kcnq1 and sarcomeric α-actinin in the same section.

Figure 2. Evaluation of the steady-state K⁺ current, I_{ss}, in ventricular myocytes isolated from adult *Kcnq1*^{+/+} and *Kcnq1*^{-/-} hearts before and after challenge with the β-adrenergic agonist, isoproterenol (Iso)

(A) Voltage-clamp protocol used to record outward K⁺ currents in these experiments. The left tracing shows control currents recorded without any drugs in normal Tyrode's solution. The tracing on the right shows I_{ss} recorded using the same voltage protocol following replacement of Tyrode's solution with "I_{ss} solution", a Tyrode's solution containing 4AP to block I_{to} and E4031 to block I_{Kr} (see Methods for details). Panels (B) and (C) show examples of I_{ss} recorded in the absence and presence of Iso for *Kcnq1*^{+/+} and *Kcnq1*^{-/-} myocytes, respectively. Note that Iso enhances I_{ss} in *Kcnq1*^{+/+} (B) but not *Kcnq1*^{-/-} (C) myocytes. (D) Comparison of

average I_{SS} densities for $Kcnq1^{+/+}$ (n=15) and $Kcnq1^{-/-}$ (n=17) myocytes in the absence (Control) versus the presence of Iso. (E) Comparison of the Iso-induced difference current (ΔI_{SS}) measured from the data sets shown in panel D. *, $p < 0.05$ using Student's T-test for statistical evaluation of significance.